a model in which both systemic arterial and IC bFGF effectively promote coronary collateral growth, IP bFGF was not efficacious. A key difference between parenteral and IP administration is *directional* (delivery via the vascular lumen vs. abluminal delivery). This factor may be responsible for the lack of effect of IP bFGF in this study.

961-117 C-Type Natriuretic Peptide Mediates cGMP Generation and Antimitogenic Actions in Human Coronary Vascular Smooth Muscle Cells

J. Schirger, J.C. Burnett, Jr., C.-M. Wei. *Mayo Clinic, Rochester, MN, USA*

C-type natriuretic peptide (CNP) is an endothelial cell derived cardiovascular peptide which mediates vasodilatory and antimitogenic actions. Previous studies demonstrate that the biological actions of CNP are mediated through the activation of a particulate guanylyl cyclase receptor and cGMP. To date, it remains unclear as to the ability of CNP to stimulate cGMP generation and to inhibit proliferation in human coronary vascular smooth muscle cells (HCVSMCs). Therefore, the present studies were designed to determine 1) the effects of CNP upon cGMP generation in HCVSMCs and 2) the antimitogenic actions of CNP upon endothelin-1 (ET-1) mediated proliferation in HCVSMCs. We hypothesized that CNP increases cGMP generation in HCVSMCs and inhibits the proliferative effect of ET-1 in HCVSMCs. To test this hypothesis we determined cGMP by radioimmunoassay in cultured HCVSMCs in the presence and absence of CNP. Additionally, cultured cells were stimulated by ET-1 (10-7M) in the presence and absence of CNP (10-7M) and thymidine incorporation was determined.

cGMP (pmol/ml)	2 hours	8 hours	24 hours
Control	0.50 ± 0.10	0.45 ± 0.05	0.65 ± 0.15
CNP (10-7M)	0.95 ± 0.55	1.35 ± 0.45	2.80 ± 1.00

ET-1 increased thymidine incorporation in HCVSMCs as compared to unstimulated cells (1362 ± 288 vs 190 ± 24 cpm/well, $p < 0.05$) and CNP inhibited ET-1 mediate thymidine incorporation (296 ± 51 vs 1362 ± 288 cpm/well, $p < 0.05$). These studies support the concept that CNP is an important activator of cGMP accumulation in human coronary vascular smooth muscle cells and plays an important autocrine and paracrine role as an inhibitor of mitogen-mediated proliferation of HCVSMCs.

961-118 Basic Fibroblast Growth Factor is a Coronary Vasoconstrictor in Experimental Hypercholesterolemia

D. Hasdai, V. Mathew, R.S. Schwartz, D.R. Holmes, Jr., A. Lerman. *Mayo Clinic, Rochester, MN, USA*

Basic fibroblast growth factor (bFGF) is a vasodilator which is dependent on the endothelium-derived relaxing factor (EDRF) pathway. Hypercholesterolemia (HC) is characterized by decreased coronary EDRF activity. Thus, we examined whether the reduced EDRF activity associated with HC results in coronary vasoconstriction in response to bFGF. Intracoronary bFGF (0.02 μg/kg/min) was infused at baseline and after 10 weeks of high-cholesterol diet. In addition, L-N^G-momo-methyl-arginine (L-NMMA), a nitric oxide synthase inhibitor, was infused intracoronary at baseline 5 (μg/kg/min) with and without bFGF. Percent change in coronary artery diameter (%ΔCAD) and coronary blood flow (%ΔCBF) were calculated based on coronary quantitative angiography and Doppler: *P < 0.05 vs. baseline, †p < 0.05 vs LNMMA and baseline bFGF.

	bFGF (n = 5) Baseline	bFGF (n = 5) 10 wks. HC	LNMMA (n = 4)	LNMMA + bFGF (n = 7)
% ΔCAD	12 ± 5	−14 ± 6*	−21 ± 4	−75 ± 12†
% ΔCBF	33 ± 33	−45 ± 8*	45 ± 5	−89 ± 9†

Conclusions: BFGF is a mild coronary vasodilator in the steady-state. However, endogenous (HC) and exogenous (by L-NMMA) inhibition of EDRF activity resulted in coronary vasoconstriction in response to bFGF. These studies suggest that the coronary vasomotor effects of bFGF are regulated by the status of the EDRF pathway.

961-119 Secretion of Brain Natriuretic Peptide in Cultured Human Coronary Vascular Smooth Muscle Cells: A New Autocrine and Paracrine Role for the Natriuretic Peptide System

C.-M. Wei, D.M. Heublein, J.C. Burnett, Jr.. *Cardiorenal Research Laboratory, Mayo Clinic, Rochester, MN, USA*

Brain natriuretic peptide (BNP) is a peptide of cardiac origin which regulates plasma volume and also vascular tone and growth. Recently, we have reported that BNP is a potent inhibitor of endothelin-1 mediated proliferation in human coronary vascular smooth muscle cells (HCoVSMC). While BNP has been reported to be produced and released from atrial and ventricular myocardium, we investigated the hypothesis that BNP may be present and secreted from human coronary vascular smooth muscle cells. Therefore, the present study was designed to investigate the secretion of BNP in cultured human coronary vascular smooth muscle cells (HCoVSMC: Clonetics, San Diego, CA). BNP and its second messenger cGMP in culture media were determined by radioimmunoassay. The presence of BNP was determined by immunohistochemical staining using a human BNP polyclonal antibody. BNP immunoreactivity was significantly increased in culture media after 48 hours incubation compared with baseline (323 ± 85 vs 11 ± 4 pg/ml, $p < 0.05$). Cyclic GMP immunoreactivity also increased in coronary vascular smooth muscle cells after culture (0.4 ± 0.03 vs 0.05 ± 0.01 pmol/min, $p < 0.05$). To confirm the presence of BNP and cGMP in HCoVSMC, two stage immunohistochemical staining was performed. In these studies, BNP and cGMP stained positively in cytoplasm of HCoVSMC while non-immune staining was negative. The current study demonstrates that BNP is secreted by cultured HCoVSMC in association with an increase in cGMP accumulation. These data suggest a probable autocrine and paracrine role for BNP as a peptide of coronary vascular smooth muscle origin which may participate in coronary vascular smooth muscle regulation.

961-120 Effect of 17β-Estradiol Treatment on Intimal Apoptosis and Cell Proliferation in an Experimental Atherosclerosis Model

J. Kamenz, H. Hanke, S. Hanke, C. Lenz, H. Kahn, V. Hombach. *University of Ulm Medical Center, Div. of Cardiology, Ulm, Germany*

It has been shown that administration of 17β-Estradiol does reduce intimal thickening in experimental atherosclerosis. So far the mechanism of the atheroprotective effect of estrogen is not well understood. Aim of the present study was to determine the effect of estrogen on intimal apoptosis in comparison to cellular proliferation during plaque development in an experimental atherosclerosis model. Thirty female New-Zealand rabbits were included in this study. Six rabbits served as a control group without cholesterol feeding (CHOL) or hormone treatment. Twenty-four rabbits received a 0.5% CHOL diet for 12 weeks and were separated in 3 different groups:

Group A only CHOL, *Group B* CHOL + ovarectomy, *Group C* CHOL + ovarectomy + 17β-Estradiol 1 mg/kg BW/week for 12 weeks. The aortic arch was then histologically and morphometrically analyzed. For in-situ detection of apoptosis the TUNEL-technique (TdT-mediated dUTP nick end labeling) was used. Bromodeoxyuridine-labeling allowed the determination of cells undergoing DNA-synthesis. *Results:*

Study group	Intimal area (mm^2)	Apoptotic cells/mm^2	Proliferating cells/mm^2
Control n = 6	< 0.1	0	0
Group A n = 8	4.5 ± 2.8	4.4 ± 1.7	46.7 ± 16.3
Group B n = 8	4.8 ± 1.7	3.7 ± 1.5	45.6 ± 11.9
Group C n = 8	1.4 ± 0.9 ($p < 0.05$)	6.1 ± 4.3	21.6 ± 22.6 ($p < 0.05$)

In summary, continuous estrogen treatment during atherosclerotic plaque development seems to reduce intimal cell proliferation and to have no influence on the apoptosis rate in the intima, resulting in a significantly smaller intimal area.

961-121 Association of Transforming Growth Factor-beta Isoforms with Coronary Plaque in Patients with Coronary Ischemia

H. Ueda, M. Imazu, K. Sumii, H. Yamamoto, K. Ono, F. Tadehara, Y. Hayashi, W. Yasui, M. Yamakido. *Hiroshima University School of Medicine, Hiroshima, Japan*

Transforming growth factor (TGF) -βs are important cytokines in the vascular system. But an association between their expression in coronary lesions and clinical pathophysiology has not been fully elucidated. We studied specimens of coronary lesions obtained at directional coronary atherectomy in 59 patients with the following diagnoses: stable angina (SA, n = 8), acute coronary syndromes (ACS, n = 21), or restenosis (RS, n = 30). The specimens

	SA (n = 8)	ACS (n = 21)	RS (n = 30)
TGF-β1	0.75 ± 0.16	1.81 ± 0.25*	1.60 ± 0.18*
TGF-β2	1.13 ± 0.30	2.24 ± 0.17*†	1.40 ± 0.15
TGF-β3	1.63 ± 0.26	2.38 ± 0.16*	2.27 ± 0.13*
TβR-I	2.38 ± 0.18	2.38 ± 0.16	2.63 ± 0.10
TβR-II	2.13 ± 0.23	2.05 ± 0.19	2.32 ± 0.13
Intimal hyperplasia	38%	71%	90%*
Neovascularization	13%	57%*	40%

* $p < 0.05$ vs. SA, † $p < 0.05$ vs. RS

were immunohistochemically analyzed for the expression and localization of TGF-β (TGF-β1, -β2, and -β3) and its receptor (TβR-I and -II) isoforms in coronary plaques (graded from 0 to 3).

Immunoreactivity for TGF-βs and TβRs was observed in the cytoplasm of smooth muscle cells (α-actin positive), nonfoamy macrophages (CD68 positive) and endothelial cells (CD31 positive). High numbers of TGF-β and TβR-positive cells were seen in intimal hyperplasia (IH) and neovascularized lesions with hemorrhage or inflammation. Moreover, in IH, the expression level of each TGP-β isoform varied. In conclusion, our results suggest that TGF-β isoforms are involved in the pathogenesis of coronary lesions in acute coronary syndromes and restenosis, and that they have specific functions in the coronary plaque formation.

962 Microvascular, Thrombotic, and Spastic Angina

Monday, March 17, 1997, 3:00 p.m.–5:00 p.m.
Anaheim Convention Center, Hall E
Presentation Hour: 3:00 p.m.–4:00 p.m.

MONDAY POSTER

962-90 Exercise Induced Changes of Fibrinolytic and Thrombotic Potential in Runners Following the 100th Boston Marathon

J.J. Stec, G.H. Tofler, I. Lipinska, P.M. Ridker, A.J. Siegel. *Harvard Medical School, Boston, MA, USA*

Although moderate physical activity is cardioprotective, strenuous exertion has been occasionally associated with the triggering of acute myocardial infarction (AMI). Since thrombosis plays an important pathogenetic role in the onset of AMI, we examined the effect of marathon running on the fibrinolytic and thrombotic potential in 24 healthy male participants, mean age 50 ± 10 years. The subjects had blood drawn the morning before and within 5 hours following the 100th Boston Marathon. Values are mean ± standard error of mean. Paired t-tests were performed.

	Before	After	p-value
Fibrinolytic activity (mm^2)	72 ± 11	173 ± 14	0.001
D-Dimer (ng/ml)	180 ± 28	405 ± 42	0.001
von Willebrand factor (%)	89 ± 9	210 ± 14	0.001
C-reactive protein (ng/ml)	147 ± 35	650 ± 110	0.001
Fibrinogen (mg/dl)	244 ± 10	234 ± 9	0.03

Marathon running resulted in a significant increase in fibrinolytic activity and D-Dimer, von Willebrand factor and C-reactive protein. Fibrinogen levels were slightly reduced.

In these healthy participants activation occurred of both fibrinolysis and prothrombotic factors suggesting a hemostatic balance. Further studies are needed to determine the effect of prolonged strenuous exercise on thrombotic potential, particularly in subjects at increased cardiovascular risk.

962-91 Attenuated Success Rate of Thrombolysis for Acute Myocardial Infarction by Reduced Endogenous Fibrinolysis

T.K. Nordt, M. Moser, B. Kohler, K. Peter, C. Bode. *Med. Universitätsklinik Heidelberg, Germany*

Resistance to thrombolysis in acute myocardial infarction (AMI) constitutes a major limitation of this form of therapy. To delineate the contribution of the various components of the blood coagulation system (i.e. platelet function, thrombin activity, and endogenous fibrinolytic activity), platelet aggregation (induced by 2 μM ADP), thrombin antithrombin complexes (TAT, measured by ELISA), and plasminogen activator inhibitor type-1 (PAI-1, measured both by ELISA and by using chromogenic substrate S-2251) were determined in 31 patients with AMI at 0, 1, and 2 h after initiation of thrombolytic therapy. Patients were treated with fibrin-specific plasminogen activators (t-PA, r-PA), patency was assessed by coronary angiography at 1.5 h and graded according to the TIMI criteria. With respect to platelet aggregation (both slope and maximum) and TAT levels there was no significant association with the TIMI grade. TAT levels at 0 h even tended to be higher in successfully treated patients: 2.1 ± 0.0 ng/ml with TIMI grade 0, 1.6 ± 0.2 with I, 5.2 ± 2.1 with II, and 5.0 ± 1.3 with III (± SEM). However, plasma concentrations of PAI-1 were significantly increased in patients with unsuccessful outcome in comparison to those with successful treatment: 39 ± 1 ng/ml with TIMI grade 0, 31 ± 7 with I, 30 ± 7 with II, and 24 ± 3 with III (± SEM, $p < 0.05$). Plasma activity of PAI-1 measured before thrombolysis showed parallel results. No difference could be found for the thrombolytic agent used. Thus, resistance to thrombolytic therapy in AMI appears to be caused by reduced endogenous fibrinolysis secondary to increased PAI-1 in plasma rather than by increased platelet or thrombin activity. Patients with elevated baseline PAI-1 may profit more consistently from alternative reperfusion strategies.

962-92 Abnormal Microvascular Responses in Cardiac Syndrome X Cannot Be Explained Solely By The Presence of Epicardial Subangiographic Atheroma

I.D. Cox, J.R. Clague, J.P. Bagger, D.E. Ward, J.C. Kaski. *St. George's Hospital, London, UK*

Aim: To study the relationship between coronary microvascular responses and epicardial sub-angiographic atheroma (SAA) in syndrome X.

Patients: We studied 9 patients (8 women; median age 55, range 43–72) with syndrome X (anginal pain, positive exercise ECG and normal coronary angiography) who were undergoing repeat coronary angiography due to persistent disabling anginal pain despite anti-anginal therapy. None had documented coronary spasm, blood pressure > 150/95 mmHg, left ventricular hypertrophy or cholesterol > 6.5 mmol/l, and all were non-smokers.

Methods: Vasoactive drugs were stopped ≥5 half-lives prior to catheterisation. Repeat angiography was completely normal in all cases. Coronary flow velocity responses during intracoronary infusion of acetylcholine (ACh – 0, 10^{-7} and 10^{-6}M) and following an intracoronary bolus of 300 μg glyceryl trinitrate (GTN) were assessed using a Doppler ultrasound guide-wire deployed in the proximal LAD artery. Simultaneous quantitative angiography was performed to enable calculation of coronary flow volume reserve i.e. the ratio of baseline to peak flow volume for each dilator (CFR-ACh/GTN). Intimal thickening due to SAA was measured by intravascular ultrasound at 1 mm intervals through the LAD.

Results: The median CFR-ACh was 1.76 (range 1.3–4.5) and was classified as abnormal (< 2) in 5 patients; CFR-GTN was > 2 in all cases. Six patients had significant intimal thickening (> 0.3 mm) and the median maximal intimal thickness was 1.0 mm (range 0–1.2 mm) Two of the five patients with CFR-ACh < 2 had no significant SAA and there was no significant correlation between the severity of SAA and CFR-ACh (rho = 0.054, p = NS; Spearman's Rank Correlation Coefficient).

Conclusions: Abnormal endothelium-dependent microvascular responses in syndrome X patients cannot be explained simply by the presence of SAA.

962-93 The Impact of Coronary Flow Reserve and Insulin Resistance on Myocardial Energy Metabolism in Syndrome X

H.E. Bøtker, H.S. Sonne, J.P. Bagger, T.T. Nielsen. *Department of Cardiology, Skejby University Hospital, Aarhus, Denmark*

It remains controversial whether myocardial ischemia is present in patients with syndrome X. Myocardial energy efficiency may be compromised due to an attenuated increment of carbohydrate oxidation during pace stress. To evaluate the influence of coronary flow reserve and myocardial substrate uptake on cardiac energy efficiency, we used coronary sinus catheterization and studied myocardial metabolism at rest and during pace stress in 18 consecutive patients with syndrome X and in 10 control subject (C). The patients were subclassified as microvascular angina (MA, n = 8) in the presence of a reduced coronary flow reserve (< 2.5) or no microvascular angina (non-MA, n = 10) if coronary flow reserve was preserved (≥2.5). *Results:* Patients with non-MA revealed fasting hyperinsulinemia (55 ± 5 vs. MA: 26 ± 5 and C: 29 ± 2 pmol × l^{-1}, $p < 0.05$) and increased arterial concentration of free fatty acids (FFA) (1.41 ± 0.19 vs. MA: 1.22 ± 0.15 and C: 1.01 ± 0.12 mmol × l^{-1}, $p < 0.05$). During pacing, myocardial uptake of FFA was increased in the non-MA patients. Net myocardial lactate release was not observed in any patients. In all subjects, myocardial FFA uptake correlated positively while glucose uptake correlated inversely with arterial concentrations of FFA. Myocardial energy expenditure, and net carbohydrate- and lipid oxidation were similar in the three groups. *Conclusion:* Not only angina pectoris and ST-segment depression but also a decreased coronary flow reserve and insulin resistance, associated with a relatively increased myocardial FFA uptake, coexist with preserved myocardial energy efficiency in patients with syndrome X.